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Detection of Heavy Metal Accumulation and Potential Health Risk Assessment of Selected Fish Species from Kolong River, Assam, India

Bhabana Das, Manash Pratim Borah*, Lalit Mohan Goswami and Priyanki Sharma

Department of Zoology, Nowgong College (Autonomous), Nagaon – 782001, Assam, India; manashpborah30@gmail.com

Abstract

The Kolong river is situated in the district of Nagaon in the northeastern state of Assam, India. This is one of the most polluted rivers in India. Fish is a prime source of protein for the people living alongside the river, making it important to determine if the consumption of fish from the Kolong river is safe or not. Two freshwater fishes, *Labeo rohita* and *Puntius conchonius* were collected from the Kolong river and analysed for the concentration of heavy metals Arsenic, Zinc, Cadmium, Chromium, Mercury and Lead (As, Zn, Cd, Cr, Hg and Pb) by Atomic Absorption Spectrophotometry. Out of the six heavy metals analysed, only three (Cd, As and Zn) were detected in the selected fish species. *Puntius conchonius* showed a low concentration of heavy metals as compared to *Labeo rohita*. All the heavy metals detected in *Labeo rohita* were found to be highest in the brain followed by the gills. Detected heavy metals were below the permissible limit in all the fish samples. An assessment was conducted on the potential health risks due to consumption of these fish species detected with heavy metals, through estimation of Metal Pollution Index (MPI), Estimated Daily Intake (EDI), Target Hazard Quotient (THQ) and Carcinogenic Risk (CR). The estimated values of MPI, EDI, THQ and CR indicated that the consumption of these selected fish species of Kolong river is safe for the adult population. However, the As level was found to be higher in the brain and gills of *Labeo rohita*, so it is highly recommended to discard these organs before consumption.

Keywords: *Labeo rohita*, Pollution Index, Proximate Analysis, *Puntius conchonius*

1. Introduction

The Kolong river, situated in Nagaon district in the northeastern state of Assam, India, is a 230km long tributary of the mighty river Brahmaputra. It runs along three districts of Assam, namely Nagaon, Marigaon and Kamrup[1]. The present study is based in the area of the river covering the district of Nagaon. Human settlements on both sides of the river have led to various anthropogenic activities like burning domestic waste and fossil fuels and discarding domestic garbage into the river or on the banks of the river, which might include medical waste, sanitary waste or any other kinds of harmful solid waste and concoction of hazardous industrial waste with the river water due to the ongoing construction ventures in the vicinity of the Kolong river. As a result of these activities, pollution in the Kolong river is observable to any passing spectator. As a result, the Kolong river is listed as one of the 71 most polluted rivers in India[2].

**Author for correspondence*

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Fish species inhabiting polluted water bodies like the Kolong river face the risk of accumulating heavy metals. Heavy metals are non-essential elements that, when accumulated in fish, might upheaval many physiological mechanisms, including cellular growth and differentiation, regeneration of damaged cells, caspase-mediated cell death and necrosis. The elements categorised as 'heavy metals' are arsenic, cadmium, chromium, mercury and lead[3,4]. Fish may accumulate these heavy metals through physical contact with the polluted waters or by intake of contaminated waters during respiration or ingestion[5]. The affinity for the accumulation of heavy metals in fish tissues differs from metal to metal based upon the total amount of metal present, the total duration of exposure, the metal uptake mechanism and environmental parameters like temperature, hardness, salinity and pH of the water, and internal characteristics like the age and nutritional behaviour of the fish species[6]. Accumulation of toxic heavy metals in different tissues like the liver, gills, brain and muscle can excessively impact the well-being of physical and chemical processes in fish[7]. Based upon the amount of metal content in the river, the length of its exposure, and the kinds of fish species, heavy metals may come across as carcinogenic, genotoxic agents or disruptors of the reproductive systems of the fish[8,9].

Cadmium (Cd) is a naturally non-biodegradable and tenacious heavy metal linked to a wide range of human ventures like cosmetics, tiles, plastics, glass, tyres of vehicles and other synthetics[10-12]. Arsenic (As) is categorised as a Class I human carcinogen that circulates in water weathering reactions, microbiological affairs, geochemical processes and other human endeavors[13,14]. As's toxicity includes everything from skin lesions to cancer of different organs like the brain, liver, kidney, and stomach, thus harming human health to quite a distinctive level[15]. Another heavy metal named Mercury (Hg) accumulates in aquatic life forms in the form of sulphide[16]. Lead (Pb) is a non-biologically hazardous heavy metal with no recognised physiological contribution in animals or humans. On the contrary, it affects the central nervous system and causes severe health issues in animals[17,18]. India is abundant in freshwater bodies and Cr is a prevalent pollutant in these water bodies, which contaminates the inhabitant edible fish in excessive quantities on a regular basis[19]. Freshwater fish require a specific amount of Zinc (Zn) for their overall development and the proper functioning of their physiological mechanisms, but long-term and persistent exposure to the same can lead to pathogenic damage to their tissues. As far as human health is concerned, consumption of Zn-accumulated fish can even cause Alzheimer's disease[20].

An abundance of research has been conducted on the determination of heavy metals in rivers as well as on fish species globally and in other states of India. However, in the northeastern states of India, this area of research was observed to be limited. Especially in Assam, where the present study is based, no past works have been done as such to determine the concentration of heavy metals in different organs of any fish species. The studies are limited to the detection of metals in the water or the sediment of contaminated water bodies (rivers, estuaries, wetlands). This is an unexplored area that holds immense potential for research purposes. Nagaon is a thickly populated district with a population of 28.23 lakh[21] and demands a high rate of protein sources for nutrition, with fish being one of their prime sources of protein. One can gain profound health benefits from the consumption of protein-rich fish, but the subsequent bioaccumulation of heavy metals can lead to potential health risks in humans[22].

The impact of heavy metals on the water ecosystem has turned out to be a major global concern[23] and demands extensive research on the detection of different heavy metals in all the possible fish species that inhabit the river. Heavy metals reach the aquatic food chain through ingestion of food and water, passing through either the digestive tract or non-dietary pathways through permeabilised membranes such as the muscles and gills[24]. Hence, the detection of heavy metals and analysis of the nutrient content was done on the gills, liver, muscle and brain of both the selected fish species, along with an assessment of possible health risks to humans due to the consumption of fish accumulated with heavy metals, to determine if the consumption of fish from the Kolong river is safe or not.

2. Materials and Methods

2.1 Collection of Samples

The study area of the Kolong river is located in the district of Nagaon, Assam. 10 samples of two fish species, *Labeo rohita* and *Puntius conchonius*, were collected from two different locations in the Kolong river. *Labeo rohita* and *Puntius conchonius* were selected for the study as these are commonly found fish species in the Kolong river. The two locations were selected based on convenience and

feasibility. Fishing nets were used for catching fish. No ethical grounds were breached during the collection of the fish, as these were found in abundance in the river and fishermen regularly catch these fish to sell in the market. The live fish samples were put into water-filled containers and transferred carefully to the laboratory. The fish samples were weighed and measured. The fish samples were dissected and multiple organs like the brain, liver, gills and muscle tissue were taken out for various proximate analyses.

2.2 Proximate Analysis

The amount of carbohydrates in the different organ samples of *Labeo rohita* and *Puntius conchonius* was determined by following Dreywood's Anthrone method[25].

The amount of protein in the different organ samples of *Labeo rohita* and *Puntius conchonius* was determined by following Lowry's method[26].

The weights of fresh tissue in all the fish samples were recorded before being dried in the oven in pre-weighed petri dishes. The dried tissues were weighed, and the percentage of moisture content was determined by the formula[27]:

$$Moisture\% = \frac{Fresh\,Tissue - Dried\,Tissue}{Fresh\,Tissue} \times 100$$

The lipid content in the fish samples was estimated by the Folch method[28].

2.3 Heavy Metal Analysis

2.3.1 Wet digestion

The fish were dissected and the brain, liver, gills and muscle tissue of both fish species were removed and analysed separately. 1g of each sample was homogenised and digested with concentrated nitric and hydrochloric acid in a ratio of 3:1. The samples were heated for 2 hours at 60°C in the water bath until the colour changed. The resulting solution mixture was cooled, filtered into a 100-ml flask and brought to the proper consistency using distilled water[29].

2.3.2 Atomic Absorption Spectrophotometry

Atomic Absorption Spectrophotometry (AAS) is a standard analytical technique for detecting element concentrations in a sample. The heavy metals (Cr, Cd, As, Hg, Pb and Zn) were determined using flame furnace AAS.

2.3.3 Statistical Data Analysis

The data obtained was analysed statistically with the help of the Data Analysis Tool Pack available on MS Excel. The significance of the difference between the nutritional content and the concentration of the analysed heavy metals in the entire organ sample from *Labeo rohita* and *Puntius conchonius* was assessed using a t-test.

2.4 Health Hazard Estimation

2.4.1 Metal Pollution Index (MPI)

The MPI was determined to compare the total metal contents present in the tissue samples of the selected fish species taken under examination. The MPI describes the level of heavy metal contamination in fish tissue. It is established that a higher MPI value indicates a higher level of contamination in fish[30].

The MPI value was calculated using the formula: MPI = (M1×M2×M3×…×Mn) 1/n, where Mn is the metal content expressed in microgram per gram of wet weight[31].

2.4.2 Estimated Daily Intake (EDI) and Recommended Daily Allowance (RDA)

To determine if the metal concentration detected in the selected fish samples from the Kolong river was safe for human consumption (adults and children), the EDI and RDA values were estimated and compared with one another. This study solely looked at the fish muscle, as the majority of people consume this portion. The EDI was calculated using the following formula:

EDI = DFC x MC, where, DFC = Daily Food (fish) consumption, MC = Mean Concentration of metal in fish sample. The daily consumption rate of fish for an adult (60kg) was an average of 49.5 g on a fresh weight basis[32].

2.4.3 Carcinogenic Risk

Carcinogenic Risk (CR) refers to the gradual and lifetime probability of an individual developing cancer from exposure to any potential carcinogenic substances. The Cancer Slope Factor (CSF) published by the United States Environmental Protection Agency (USEPA) was considered to determine the cancer risk over a lifetime exposure to Cd, Zn and As. The formula for CR is CR =

CSF x EDI, where CSF is the carcinogenic slope factor, which is 0.38 for Cd and 1.5 (mg/g/day) for As[33].

2.4.4 Target Hazard Quotient (THQ)

The THQ is used to determine the non-carcinogenic risk level due to pollutant exposure. The evaluation of the health risk from metal-contaminated fish species in the Kolong river was done through the determination of the THQ value as per the USEPA Region III Risk-Based Concentration Table[33] by using the following equation:

$$THQ = \frac{MC \times IR \times EF \times ED \times CF \times 10^3}{RfD \times BW \times ATn}$$

Here, MC = Total concentration of heavy metal (mg/g d.w.), IR = Rate of fish ingestion (0.02g/person/day for adults), EF = Exposure Frequency (365 days/year), ED = Exposure Duration (70 years); CF = Factor for the conversion of fresh weight to dry weight by considering 79% of moisture content (0.208), RfD = Reference dose of individual metal (Cd is 0.05 mg/g, As is 0.12 mg/g and Zn is0.5 mg/g), BW = Average body weight of an adult (70 Kg) and ATn = Average exposure time for non-carcinogens (10,950 days)[34].

3. Results and Discussion

The site of study, Kolong river, Nagaon, Assam has been in a moribund state due to pollution as shown in Figure 1.

Two fish species, *Labeo rohita and Puntius conchonius* were selected from the Kolong river for the study. *L. rohita* is a column feeder whereas *P. conchonius* is a surface feeder (Figures 2 and 3). Their body length and weight also differ from each other (Table 1).

The length of the *L. rohita* ranged from 13 to 13.5cm and its weight ranged from 22.7 to 29.3 grams. *P. conchonius* was shorter and smaller as compared to *L. rohita*, as the samples of *P. conchonius* varied from 6.5 to 9cm in length and 5.6 to 11.9 grams in weight.

3.1 Analysis of Proximate Composition

The proximate composition of four different organ tissues, i.e., the brain, liver, gills and muscles, of both the fish species were analysed to determine their nutritive value. It was observed that in *L. rohita*, the concentration of carbohydrates increases in the order from gills to muscles, while in *P. conchonius*, the concentration of carbohydrates increases in the order from brain to liver (Table 2). In both the fish species, the liver and muscles



Figure 1. Environment in and around river Kolong: **(A)** Anthropogenic activities like washing clothes, bathing etc. **(B)** Dumping of garbage **(C)** Fishing amidst polluted waters **(D)** Algal bloom.

Table 1. Information on the selected fish species of Kolong river

Common Name	Scientific Name	Average Length (cm) (Mean±SD)	Average Weight (grams) (Mean±SD)
Rohu	*Labeo rohita*	13.5 ± 0.058	27.002 ± 3.728
Puthi	*Puntius conchonius*	7.5 ± 1.323	9.76 ± 3.608

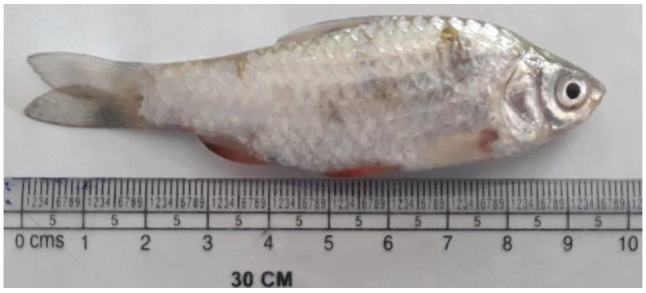


Figure 2. *Punctius conchonius.*

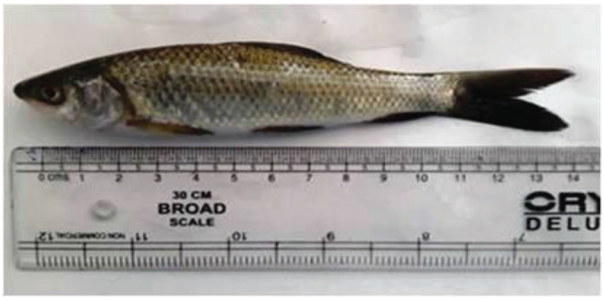


Figure 3 *Labeo rohita.*

contain a substantial amount of carbohydrates, which is justified by previously done studies which stated that when the body breaks down most carbohydrates from the foods we eat, they are converted into a type of sugar called glucose and stored in the liver and muscles[35]. The protein concentration in *L. rohita* and *P. conchonius* is highest in the liver (0.245±0.001 mg/g) and (0.391±0.006 mg/g), respectively. The liver remains a common organ, containing a high amount of both carbohydrates and protein. Several studies have earlier found similar results of the liver being a good source of protein containing a high nutritive value[36]. In *L. rohita*, the moisture content is highest in the gills (85.25±0.29%) and lowest in the brain (71.47±0.46%), while in *P. conchonius*, the brain (74.71±0.55%) had the highest moisture content and the liver (61.49±0.71%) the lowest. The lipid concentration in *L. rohita* is highest in the liver (0.68±0.01%) and brain (0.66 ± 0.058%), and in *P. conchonius* in the brain (1.66±0.055%). The brain tissue of *P. conchonius* contained the highest amount of lipid, while that of *L. rohita* contained the second highest amount of lipid. The results align with other research which states that lipids and lipid intermediates are important constituents of the structure and function of the brain. 50% of the dry weight of the brain is composed of lipids[37].

The p-values were found to be <0.05 in all the sources of variance except for the protein content between the liver and muscle of *L. rohita* with a p-value of 0.287(>0.05), the moisture content between the liver and brain of *L. rohita* with a p-value of 0.301 (>0.05) and the protein content between the gills and brain of *P. conchonius* with

Table 2. Proximate analysis in different organs of the selected fish species of Kolong river

Species	Organ sample	Concentration of carbohydrate (mg/g± SD)	Concentration of Protein (mg/g± SD)	Moisture (%±SD)	Lipid (%±SD)
Labeo rohita	Brain	1.402 ± 0.0001	0.155 ± 0.0008^{c}	71.47 ± 0.46^{a}	0.66 ± 0.058
	Liver	6.379 ± 0.027	0.245 ± 0.001^{a}	84.16 ± 0.23^{a}	0.68 ± 0.01
	Gills	0.675 ± 0.003	0.223 ± 0.001	85.25 ± 0.29	0.32 ± 0.025
	Muscle	11.178 ± 0.0015	0.156 ± 0.0007^{a}	73.02 ± 0.04	0.11 ± 0.005
Puntius conchonius	Brain	0.340 ± 0.0036	0.178 ± 0.001^{bc}	74.71 ± 0.55	1.66 ± 0.055
	Liver	7.432 ± 0.0066	0.391 ± 0.006	61.49 ± 0.71	0.52 ± 0.02
	Gills	3.402 ± 0.0548	0.199 ± 0.0007^{b}	66.08 ± 0.16	0.12 ± 0.005
	Muscle	4.165 ± 0.0006	0.198 ± 0.0005	70.74 ± 0.41	1.60 ± 0.013

*Values are shown as Mean± SD *Values with the same superscript in a column do not vary significantly (p>0.05).

a p-value of 0.391 (>0.05), which does not have significant differences between them.

The p-value of protein content between the brains of *L. rohita* and *P. conchonius* is >0.05, which implies there is no significant difference between the two samples. The carbohydrate, protein, moisture and lipid content in the liver, muscle and gills and the carbohydrate, moisture and lipid content in the brain of *P. conchonius* and the carbohydrate, protein, moisture and lipid content in the liver, muscle, gills and brain of *L. rohita* have statistical significance with a p-value <0.05.

Numerous research has been done in the past which reported an increased amount of protein in the liver. The liver is a metabolising organ that synthesises many enzymes and hence shows a higher amount of protein and carbohydrates as compared to other tissues of both the fish species. Carbohydrates have a significant role to play in cellular metabolism. It provides energy to all kinds of cells in the body. The same has been backed by evidence from previous studies which discovered the highest amount of carbohydrates in the muscle tissues of fish. In the present study too, carbohydrates were found highest in the muscle tissue of *L. rohita* but the results differed in the case of *P. conchonius* as the carbohydrates are found highest in the liver instead of muscle[38]. The inhibition of oxidative phosphorylation may be the cause behind lower lipid content in all the examined tissues of both the fish species, evidence of the same can be traced in the works of Chezhian A *et al*[39]. Earlier scientific papers mentioned that moisture is one of the fundamental components of all fish species and is present at a high concentration in all the tissues[40]. In the current study as well, the concentration of moisture ranged from 61.49±0.71 to 85.25±0.29 across all the tissue organs of the fish species, *L. rohita* and *P. conchonius*. This increased level of moisture could be a result of great accessibility to water and its active nature[38]. Studies also suggest that a higher percentage of moisture indicates a good amount of protein, carbohydrate and lipid in the tissues[38].

3.2 Analysis of Heavy Metal Concentration

The presence of the six heavy metals was analysed. The results, as shown in Table 3, imply that Cr, Pb and Hg were not detected in any of the studied organ tissues of the selected fish species, *L. rohita* and *P. conchonius*. The present results from a similar study conducted on the river Ganga found an elevated increase of Cr and Pb in the waters[41]. However, Cd, As and Zn were detected in both the fish species. Findings from prior research revealed that the location and species of fish affected the metal concentrations in their muscles, which varied greatly[5]. Literatures from past research put across the various factors which influence the concentration of metals in fish species. The age and constituents of rocks at the bottom, contamination by anthropogenic activities and soil formation are a few factors to name some[5].

Cd was found to be highest in the brain and gills (0.003±0.0001) in *L. rohita*. In *P. conchonius* muscle (0.0009±0.00) and liver (0.0008±0.00) accumulated higher concentrations of Cd as compared to gills (0.0007±0.01) and brain (0.0007±0.00). The concentrations of Cd

Table 3. Heavy metal concentrations (mg/g) at mean (±SD) levels in the various organs of selected fish species of Kolong river

Heavy metal	Fish species	Organ sample	Concentration (mg/g) (Mean±SD)
Chromium	*Labeo rohita*	Brain	ND
		Liver	ND
		Gills	ND
		Muscle	ND
	Puntius conchonius	Brain	ND
		Liver	ND
		Gills	ND
		Muscle	ND
Cadmium	*Labeo rohita*	Brain	0.003±0.0001[a]
		Liver	0.002±0.0001
		Gills	0.003±0.0001[a]
		Muscle	0.0024±0.0001
	Puntius conchonius	Brain	0.0007±0.00
		Liver	0.0008±0.00
		Gills	0.0007±0.00
		Muscle	0.0009±0.00
Arsenic	*Labeo rohita*	Brain	0.15±0.01[b]
		Liver	0.15±0.01[dx]
		Gills	0.007±0.001[b]
		Muscle	0.006±0.001[dy]
	Puntius conchonius	Brain	0.0044± 0.00
		Liver	0.0042 ±0.00[x]
		Gills	0.00478± 0.00
		Muscle	0.0043±0.00[y]

Table 3. Continued...

Heavy metal	Fish species	Organ sample	Concentration (mg/g) (Mean±SD)
Mercury	*Labeo rohita*	Brain	ND
		Liver	ND
		Gills	ND
		Muscle	ND
	Puntius conchonius	Brain	ND
		Liver	ND
		Gills	ND
		Muscle	ND
Lead	*Labeo rohita*	Brain	ND
		Liver	ND
		Gills	ND
		Muscle	ND
	Puntius conchonius	Brain	ND
		Liver	ND
		Gills	ND
		Muscle	ND
Zinc	*Labeo rohita*	Brain	0.71±0.0[c]
		Liver	0.41±0.02
		Gills	0.71±0.02[c]
		Muscle	0.52±0.02
	Puntius conchonius	Brain	0.32±0.02
		Liver	0.38±0.02
		Gills	0.35±0.02
		Muscle	0.40±0.02

*ND = Not Detected. Values with the same superscript in a column do not vary significantly (p>0.05).

were found to be higher in the species of Pearl river as compared to the present study in Kolong river[42].

As was found to be highest in the brain and liver (0.15±0.01) in *L. rohita*. In *P. conchonius*, the concentration of As was extremely low in all the organ samples, but amongst all samples, the gills (0.00478±0.00) and brain (0.0044±0.00) accumulated a higher concentration of As. A study carried out in the Karnaphuli river estuary, too found the concentration level of As to be the highest[5]. However, the same study found the concentration of Pb and As above the recommended threshold limit[5], which was not the scene for the present study. Interestingly, Pb was not at all detected in the fish species of Kolong river.

Zn was detected in the highest amount among all the studied heavy metals in both fish species. In *L. rohita*, the gills (0.71±0.02) and brain (0.71±0.00) accumulated the highest amount of Zn. In *P. conchonius,* muscle (0.40±0.02) and liver (0.38±0.02) accumulated the highest amount of Zn. In *L. rohita,* the brain and liver accumulated a higher concentration of heavy metals, but in *P. conchonius*, it was the muscles and liver. Early studies detected the highest amount of Zn in the muscle tissue which aligns with the present study in the case of *P. conchonius* but differs in *L. rohita*[43]. Zn is an essential metal which can be detected in all the cells which is true for previous studies[43] and the current study as well.

P. conchonius, a pelagic fish species, has a low concentration of heavy metals as compared to *L. rohita* which is a demersal species. Early research provides evidence that demersal species accumulate higher concentrations of metals as they settle at the bottom. It can be observed that all the detected heavy metals accumulate highest in the brain in *L. rohita*, followed by gills. It can be safely suggested to discard the brain and gills of the *L. rohita* of the Kolong river before consumption.

There is no significant difference between the As concentration in the livers and muscles of *L. rohita* and *P. conchonius* (p-value>0.05). However, there are significant differences between the Cd and Zn concentrations in the livers of *L. rohita* and *P. conchonius* (p-value<0.05). There are significant differences between the Cd, As and Zn concentrations in the muscle, gills and brain of *L. rohita* and *P. conchonius* (p-value<0.05).

3.3 Health Hazard Estimations

The permissible limits of the selected heavy metals of this study have been stated in Table 4. In the present study Cr, Pb and Hg were not detected in any of the fish species. Hence their concentration is definitely below the permissible limit of consumption and does not cause any harm to humans. The detected concentrations of Cd and Zn were also below the permissible limit of human consumption in the entire organ sample of both fish species. The permissible limit of each heavy metal has been provided by well-acclaimed specialised agencies of the United Nations, World Health Organization (WHO) and Food and Agricultural Organization (FAO).

In the case of *L. rohita*, the As concentration in the brain and gills was higher than the permissible limit of human consumption and hence should be discarded from

the fish before consumption. In the muscle and liver of *L. rohita* and all the organ tissues of *P. conchonius*, the As concentration was below the permissible limit of human consumption. An assessment was done to estimate the health risks to humans who consumed fish accumulated with heavy metals. The parameters used in the assessment are the Metal Pollution Index (MPI), Carcinogenic Risk (CR), Target Hazard Quotient (THQ), and Estimated Daily Intake (EDI).

Table 4. The permissible limit of heavy metals

Heavy Metal	Permissible limit of consumption in humans (mg/g)	References of permissible limit data
Chromium	0.05	(WHO, 2020)[44]
Cadmium	0.05	(FAO, 2003)[45]
Arsenic	0.1	(WHO, 2022)[46]
Mercury	0.5	(FAO/WHO, 2009)[47]
Lead	0.2	(FAO, 2003)[45]
Zinc	5	(FAO, 2003)[45]

In Table 5, we compare the RDA and EDI values of the detected heavy metals in the respective fish samples of the selected fish species. The EDI of Cd and Zn is less than the RDA in all the sample tissues. In the case of As, the gills, brain and liver of *L. rohita* hold an EDI greater than their RDA. EDI is significant in determining any possibility of negative impact on human health based on the RDA value of each metal. However, previous studies suggest against considering this as a permanent parameter to estimate health hazards in humans[48].

Unlike the other heavy metals, Zn is not included in the class of carcinogenic metals[52]. Hence, the CR was calculated only for As and Cd. The CR values lower than 10^{-6} indicate a negligible health risk whereas values in the range of 10^{-6} to 10^{-4} are in the acceptable belt[45]. The CR values of both Cd and As in all the organ samples were found to be <1, which indicates no potential cancer risk

Table 5. Comparison between RDA and EDI of the detected heavy metals in the selected fish species.

Fish species	Organ sample	Estimated Daily Intake (EDI)			Recommended Daily Allowance (RDA)		
		Cd	As	Zn	Cd	As	Zn
Labeo rohita	**Gills**	0.148	7.425	34.65	62 µg/day (Satarug 2017)[49]	0.3 µg/day (Virginia 2017)[50]	11000 µg/day (ODS 2022)[51]
	Brain	0.148	7.425	34.65			
	Liver	0.099	0.346	25.74			
	Muscle	0.118	0.297	19.8			
Puntius conchonius	**Gills**	0.034	1.715	16.83			
	Brain	0.034	1.715	15.345			
	Liver	0.039	1.960	19.80			
	Muscle	0.0445	2.205	18.315			

Table 6. Assessment of health hazard parameters in humans

Fish species	Organ samples	Metal Pollution	Carcinogenic Risk (CR)		Target Hazard Quotient (THQ)		
		Index (MPI)	Cd	As	Cd	As	Zn
Labeo rohita	Gills	0.089	0.013	0.6609	2.496×10^{-11}	2.6×10^{-12}	1.35×10^{-7}
	Brain	0.089	0.013	0.6609	2.496×10^{-11}	2.6×10^{-12}	1.36×10^{-7}
	Liver	0.028	0.002	0.0099	1.109×10^{-11}	5.662×10^{-11}	7.49×10^{-8}
	Muscle	0.026	0.003	0.0079	1.597×10^{-11}	4.16×10^{-11}	4.43×10^{-8}
Puntius conchonius	Gills	0.0105	0.0002	0.0071	1.358×10^{-12}	2.64×10^{-11}	3.20×10^{-8}
	Brain	0.0099	0.0002	0.0066	1.389×10^{-12}	2.237×10^{-11}	2.66×10^{-8}
	Liver	0.0108	0.0003	0.0063	1.774×10^{-12}	2.038×10^{-11}	4.43×10^{-8}
	Muscle	0.0115	0.0003	0.0064	2.246×10^{-12}	2.136×10^{-11}	3.79×10^{-8}

as of now. However, the high CR values in the brain and gills of *L. rohita* still hold cause for concern in the future.

Table 6 displays that MPI is extremely low for both the selected fish species, despite the presence of three heavy metals. On one hand, the gills (0.089) of *L. rohita* have the highest MPI, and on the other hand, the gills (0.265) of *P. conchonius* have the lowest MPI of all. The THQ of Cd, As and Zn in all the samples of *L. rohita* and *P. conchonius* are <1, which indicates that the metal uptake via consumption of the selected fish species poses no health risks in the study areas of Kolong river for the adult population. The THQ for As is the highest, which indicates the presence of As in high amounts in the Kolong river. In the present study, the data for As concentration might not impose serious health issues, but if in the future the accumulation of As continues to grow, it might cause various types of cancer in the skin, bladder, lungs, etc.

The study reveals that *L. rohita* and *P. conchonius* of the Kolong river are safely consumable fish species with a good amount of nutrient content. Since the health hazard parameters like MPI, EDI, THQ, and CR are below 1, they are expected to impose no potential health risk on humans. However, since the As concentration is found to be higher in the brain and gills of *L. rohita*, it is highly recommended to discard those organs before consumption.

4. Conclusion

The study investigates the concentration of six heavy metals in two commonly consumed fish species, *L. rohita* and *P. conchonius*, from the Kolong river and their possible implications for human health. The MPI, EDI, THQ, and CR were found to be below toxic levels (<1) in *L. rohita* and *P. conchonius* of the river Kolong. Hence, it can be concluded that these fish are safe for consumption. In comparison, *P. conchonius* is safer than *L. rohita* as being a pelagic species, it accumulates a lesser amount of heavy metals. Proximate analysis revealed that the nutrient content in both fish species is appropriate with respect to their sizes. However, the As level was found to be higher in the brain and gills of *L. rohita*, so it is highly recommended to discard these organs before consumption. Accumulation of toxins can cause disruption in the biological processes, aquatic food chains and genetic defects in the species[53] making it essential to implement measures to minimize the toxic impact of heavy metals on human health and the environment. Future research should focus on the toxicity of heavy metals, their trophic level transfer and their effect on the environment.

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